A novel subpopulation of peripheral blood mononuclear cells presents in major burn patients

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ABSTRACT

Hypertrophic scars (HTS) are generally believed to result from proliferation and activation of resident connective tissue fibroblasts after burns. To demonstrate a potential role of blood-borne cells, the peripheral blood mononuclear cells (PBMCs) and the effect of PBMCs on dermal fibroblast behavior was investigated.

Flow cytometry was used to analyze the surface and intracellular protein expression of PBMCs and fibroblasts. Transwell migration assay, enzyme-linked immunosorbent assay and real-time reverse transcription polymerase chain reaction was performed to assess fibroblast functions.

We identified a novel subpopulation of PBMCs in burn patients in vivo that appears at an early stage following major thermal injuries, which primarily express procollagen 1, leukocyte specific protein 1, CD204, toll-like receptor 4 and stromal cell-derived factor 1 (SDF-1) receptor CXCR4. In vitro, the conditioned media from burn patient PBMCs up-regulated the expression of fibrotic growth factors and extracellular matrix molecules, down-regulated antifibrotic factor decorin, enhanced cell chemotaxis and promoted cell differentiation into contractile myofibroblasts in dermal fibroblasts.

After thermal injury, this novel subpopulation of PBMCs is systemically triggered and attracted to the wounds under SDF-1/CXCR4 signaling where they appear to modulate the functions of resident connective tissue cells and thus contribute to the development of HTS.

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Article info

Keywords:
Burn injury
Fibrocytes
Macrophages
Hypertrophic scars

Article history:
Accepted 8 December 2014

Abbreviations: aSMA, alpha smooth muscle actin; P-PBMC, culture media from peripheral blood mononuclear cells of burn patients; COL-1, type 1 collagen; CTGF, connective tissue growth factor; CXCR4, CXC chemokine receptor 4; DCM, decorin; ECM, extracellular matrix; FN, fibronectin; HTS, hypertrophic scar(s); LPS, lipopolysaccharide; LSP-1, leukocyte specific protein 1; C-PBMC, culture media from peripheral blood mononuclear cells of normal individuals; PBMCs, peripheral blood mononuclear cells; SDF-1, stromal cell-derived factor 1; TBSA, total body surface area; TGF-β, transforming growth factor β; Th2, type 2 T helper cell; TLR4, Toll-like receptor 4.

http://dx.doi.org/10.1016/j.burns.2014.12.005
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1. Introduction

Hypertrophic scars (HTS) in humans that follow deep thermal injuries and severe traumas are a dermal fibroproliferative disorder where abnormal extracellular matrix (ECM) accumulation and cellular activity result in raised, red, itchy, firm and symptomatic scar tissue [1,2]. Although the molecular and cellular events that lead to HTS have been extensively studied, the pathogenesis of this condition is still not well understood. HTS are generally believed to result from proliferation and activation of resident connective tissue fibroblasts and myofibroblasts [3] which may migrate from adjacent uninjured cutaneous tissue, originate from resident pericytes and mesenchymal cells [4], or are derived from blood-borne fibroblast-like cells (fibrocytes) in the circulation [5–9].

The idea that matrix-producing cells could be derived from peripheral blood mononuclear cells (PBMCs) was suggested by Metchnikov and others [10,11]. Circulating fibrocytes identified in 1994 in the context of wound repair were described previously as a subpopulation of PBMCs and are unique bone marrow-derived mesenchymal progenitor cells that are implicated in the pathogenesis of fibrotic diseases in diverse organs including liver, lungs, skin and kidneys [12]. They express markers of leukocytes, hematopoietic progenitor cells and fibroblasts as well as a number of other markers including chemokine receptors and adhesion molecules, but do not normally express CD14 [13].

Stromal cell-derived factor 1 (SDF-1) is a small cytokine belonging to the chemokine family, which is also called CXCL12, and is often induced by pro-inflammatory stimuli such as lipopolysaccharide (LPS), tumor necrosis factor (TNF), or interleukin-1 (IL-1). It was originally identified as a bone marrow SDF [14] from stromal cells which includes immune cells, pericytes, endothelial cells, inflammatory cells and fibroblasts. SDF-1 has been found to be a potent chemotactrant for lymphocytes and monocytes in vitro and subsequently in vivo [15] and functions by binding to its receptor, CXCR4 [16]. Emerging experimental evidence of wound healing research indicates that circulating fibrocytes mobilized from the bone marrow may contribute significantly to wound healing and hypertrophic scarring [17,18]. Fibrocytes are believed to migrate into inflamed tissue, such as the wounded dermis the SDF-1/CXCR4 signaling axis. It has been shown that circulating fibrocytes can rapidly enter the sites of injuries, not only producing ECM molecules, but also regulating the functions of the surrounding cells. These cells secrete inflammatory cytokines, growth factors and chemokines, present antigens, stimulate angiogenesis, contribute to wound contraction, and synthesize collagen and fibronectin (FN) [19].

Of the PBMCs, macrophages also appear to be of fundamental importance in the development of post-burn immune dysfunction because they are the major producers of pro-inflammatory mediators and the productive capacity for these mediators is markedly enhanced following thermal injury. Studies suggest that γδ T-cells and alterations in cAMP-dependent processes in part mediate the expression of macrophage hyperactivity post-burn [20].

Thus, we hypothesize that a special subset of blood-borne cells originated in responding to injuries may recruit from circulation into wound sites through chemokine pathways in the early stages following the burns, where they interact with resident cells and lead to the development of dermal fibrosis.

2. Materials and methods

2.1. Patients

Blood samples were collected within 2 weeks following burns from four major burn patients with ≥50% total body surface area (TBSA) and six normal individuals. Normal fibroblasts were cultured from normal skin of a burn patient with 25% TBSA. Detailed demographic information of patients and control individuals is listed in Table 1. The patients were treated at the Firefighter’s Burn Treatment Unit and Outpatient Burn Clinic at the University of Alberta Hospital. The Health Research Ethics Board of the University of Alberta

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P, patient; C, control; M, male; F, female; HTS, hypertrophic scar; PBMCs, peripheral blood mononuclear cells; NA, not applicable.
Hospital approved the research protocol and informed consent was obtained before including patients in the study.

2.2. **PBMC isolation and conditioned medium preparation**

Twenty-five mL of heparinized peripheral blood was drawn from burn patients and controls. If the patient’s condition allowed, we collected the blood sample as early as possible following burn injury and the collection time is described as days post the injury. Total PBMCs were isolated by density gradient sedimentation on Lymphocyte Separation Medium (Mediatech Inc, Manassas, VA) following the manufacturer’s protocol. After washing with PBS, the PBMC pellet was resuspended in 10% FBS supplemented DMEM (Gibco Invitrogen Co, San Diego, CA) and 3 × 10⁶ cells were seeded per well in the 6-well plate for 24 h. The culture media from PBMCs of burn patients (P-PBMC) or controls (C-PBMC) were collected for further study.

2.3. **Flow cytometry analysis**

To determine the cell phenotype, PBMCs were stained in two tubes after FcR blocking directly by PerCP-conjugated mouse anti-human CD14 (Miltenyi Biotec, Auburn, CA), AF488-conjugated mouse anti-human CD45 (Cedarlane, Burlington, ON), APC-conjugated mouse anti-human CD204 (R&D Systems, Minneapolis, MN), PE-Cy7-conjugated mouse anti-human CXCR4 (BD Biosciences, Mississauga, ON) and APC-conjugated mouse anti-human Toll-like receptor 4 (TLR4) (eBiosciences, San Diego, CA). For intracellular staining, the cells were permeabilized with 0.5% saponin in PBS, and then incubated with mouse anti-human type I procollagen (COL-1) (Santa Cruz, Santa Cruz, CA) and rabbit anti-human leucocyte specific protein 1 (LSP-1) (Abnova, Walnut, CA), followed by secondary antibodies APC-Cy7-conjugated goat anti-mouse IgG1 (Santa Cruz, Santa Cruz, CA) and AF488-conjugated donkey anti-rabbit IgG (Life Technologies, Burlington, ON), respectively. To identify the differentiation of fibroblasts into myofibroblasts, dermal fibroblasts treated with conditioned media from PBMCs were fixed with 4% paraformaldehyde, permeabilized with 0.5% saponin in PBS, and then incubated with PE-conjugated mouse anti-human alpha smooth muscle actin (aSMA) antibody (R&D Systems, Minneapolis, MN). Finally, 10,000 cells were analyzed on a FACS Canto machine (BD Biosciences, San Jose, CA) with DiVa software (Becton-Dickinson, San Jose, CA). The isotype antibodies specific for each primary antibody were used to stain the cells as negative controls.

2.4. **Dermal fibroblast isolation, culture and treatment with PBMC conditioned media**

Normal dermal fibroblasts were isolated and cultured from normal skin specimen of burn patients as described in our previous work [21]. For this study, the normal skin sections were kept in DMEM and minced into small pieces less than 0.5 mm in any dimension. They were washed three times with sterile medium and distributed into 60 × 15 mm culture dishes, four pieces to a dish. A sterile glass cover slip, attached to the dish with a drop of sterile silicone grease, was used to immobilize the tissue fragments. DMEM with 10% FBS and 1% antibiotics was added to each flask and the flasks were then incubated at 37 °C in an atmosphere of 5% CO₂ in air at 95% relative humidity. The medium was replaced every 5 days. After 3 weeks, the cells, which appeared to be predominantly fibroblasts, were released from the dishes using trypsin and transferred to 75 cm² culture flasks. Once confluent, the cells were trypsinized and subcultured at a splitting ratio of 1:6. Fibroblasts at passages 3–6 were used in this study.

2.5. **Transwell migration assay**

The effect of conditioned media from burn patient PBMCs on the migration of dermal fibroblasts was determined using a transwell insert system. As described previously [17], conditioned media (P-PBMC) or control (C-PBMC) were added to individual wells of a 24-well plate. Transwell inserts with 8 μm of pore size (Millipore, Billerica, MA) were inserted into the wells and 100 μL of cell suspension (10⁵ fibroblasts) were loaded on the top of the insert membranes and incubated at 37 °C in 5% CO₂. After 4 h, any non-migrated cells were removed by scraping the upper side of the membranes. The membranes were then fixed with methanol and stained with hematoxylin. Fibroblast migration was assessed by counting the number of migrated cells in five high-power fields (HPFs) of each membrane under the microscope.

2.6. **Enzyme-linked immunosorbent assay (ELISA)**

To determine the effect of the conditioned media from burn patient PBMCs on the protein expression of wound healing related factors, 5 × 10⁴ dermal fibroblasts were seeded in each well of a 12-well plate and treated by conditioned media (P-PBMC) or control (C-PBMC) for 24 h. After washing, the cells were cultured for another 24 h and the supernatant was harvested for determination of transforming growth factor beta 1 (TGF-β1), connective tissue growth factor (CTGF) and decorin (DCN) using ELISA kits (R&D, Minneapolis, MN; Peprotech, Rocky Hill, NJ). The plates were read on a THERMOMax (Molecular Devices, Sunnyvale, CA) microplate reader at a wavelength of 450 nm for the detections of TGF-β1 and DCN, and 405 nm for CTGF. Recombinant human TGF-β1, CTGF and DCN were used as the standards. The data are displayed as pg/mL.

2.7. **Reverse transcription quantitative polymerase chain reaction (RT-qPCR)**

Total RNA was extracted from dermal fibroblasts treated with conditioned media from patient PBMCs using RNeasy spin columns (Qiagen, Mississauga, ON). First-strand cDNA was synthesized using an M-MLV First-Strand cDNA synthesis Kit (Invitrogen, Carlsbad, CA) using total RNA extract. RT-qPCR was conducted using SYBRs GREEN PCR Master Mix (ABI, Carlsbad, CA) according to the manufacturers’ recommendations. Amplification and analysis of cDNA fragments were carried out using a Step one Plus real-time PCR system (ABI Applied Biosystems, Carlsbad, CA). Amplification of the housekeeping gene hypoxanthine-guanine-phosphoribosyltransferase (HPRT) was used to normalize the results. The primers were forward 5’-GACCAGTCAACAGGGCA-3’ and
reverse 5’-ACACTTGAGGCTCTTTT-3’ for HPRT; forward 5’-ATTTCGACTAGACATTGG-3’ and reverse 5’-GGTTGGTTCGTC- TGTTC-3’ for COL-1; forward 5’-TGTTAAAACCTGGCATCTGA-3’ and reverse 5’-GTCTCTAGGAGTAGTGT-3’ for TGF-β1; forward 5’-GAGTGTGGCTGACAGAATG-3’ and reverse 5’-GGCTTAAATTCGGGTGTGA-3’ for COL-1; forward 5’-CTGGATAGATGACCAAG-3’ and reverse 5’-GGAGTGTGCACGCCAAAGAT-3’.

2.8. Statistical analysis

All experiments were done in triplicate. Results are expressed as mean ± SE (standard error). Statistical analysis was performed using T-test in Microsoft Excel. P-values ≤0.05 were considered statistically significant.

3. Results

3.1. A novel subpopulation of PBMCs emerged in burn patients

Compared to normal individuals (controls), we found a novel subset of PBMCs that emerged from burn patient blood at early stage following major burns using flow cytometry. They are CD14+ COL-1+. Fig. 1A and B are the representative images of flow cytometry charts. The mean percentage of total PBMCs is 16.40 ± 2.01% in burn patients and 2.55 ± 0.63% in controls (Fig. 1C).

To further identify additional markers, these cells were gated and analyzed for the expression of LSP-1, CD45, CD204, CXCR4 and TLR4. Fig. 2A shows the representative images of flow cytometry analysis. The mean percentage of LSP-1-, CD45-, CD204-, CXCR4- and TLR4-expressing cells in this subset is summarized in Fig. 2B. Of these cells, 100% express LSP-1 and CD45, 80% express CD204, suggesting a mixture of fibrocytes and immature M2 macrophages and over 70% express CXCR4 and TLR4, revealing that SDF-1/CXCR4 signaling and TLR4 pathway may be involved in the burn wound healing process.

3.2. Conditioned media from burn patient PBMCs upregulated the gene expressions of profibrotic factors and down-regulated antifibrotic factors in dermal fibroblasts

The gene expression of growth factors such as TGF-β1 and CTGF, ECM components like COL-1, FN and DCN as well as αSMA, which is an encoded protein and a marker of myofibroblasts, were quantified by real time RT-qPCR in dermal fibroblasts treated with conditioned media from PBMCs for 24 h. As shown in Fig. 3, compared to control media, the media from burn patient PBMCs significantly upregulated the gene expressions of COL-1 (fold 2.19 ± 0.27 vs

Fig. 1 – A subpopulation of PBMCs (CD14 + COL-1) in the early stage of burns in the patients. Five peripheral blood samples were collected within 2 weeks following burns from four patients and another six samples from six healthy individuals (controls). The PBMCs were isolated, stained for CD14 and COL-1 by fluorescence-conjugated antibodies, and analyzed by flow cytometry. (A and B) Representative images of total PBMCs gated in P1 and the cells-expressing CD14 + COL-1+ gated in P2. (C) The number of cells expressing CD14 + COL-1+ is displayed as percentage of total PBMCs in patients and controls and summarized in the graph. ** P < 0.001.
1.02 ± 0.14), CTGF (fold 2.65 ± 0.74 vs 1.01 ± 0.05), TGF-β1 (fold 2.04 ± 0.47 vs 1.03 ± 0.10), FN (fold 2.74 ± 0.41 vs 1.03 ± 0.10) and αSMA (fold 2.60 ± 0.41 vs 1.01 ± 0.08). However, DCN, a known antifibrotic factor was reduced in the cells treated by media from burn patient PBMCs compared to control media (fold 0.49 ± 0.07 vs 1.07 ± 0.11).

3.3. Conditioned media from burn patient PBMCs enhanced the chemotaxis of dermal fibroblasts

Fibroblast migration from around the wound site is an important landmark of wound healing following burn injury and the persistence of fibroblasts in the wound bed has been linked to fibrotic conditions and scar formation [22]. In vitro, using the transwell insert system, we tested the effect of conditioned media from patient PBMCs on fibroblast chemotaxis. Fig. 4A and B are representative images of migrated cells in the insert membranes. Fig. 4C shows that fibroblasts treated by the media from burn patient PBMCs had significantly enhanced migration compared to the fibroblasts treated with control media (290.3 ± 2.4 vs 103.0 ± 6.6 migrated cells in 5HPFs), indicating that the culture medium from burn patient PBMCs may contain chemoattractant factors that up-regulate the dermal fibroblast mobility.

3.4. Conditioned media from burn patient PBMCs promoted fibroblast differentiation into contractile myofibroblasts

Myofibroblasts play a key role in the wound healing process, including wound closure and matrix deposition, particularly, in HTS that develop following burn injury, where an increased number of myofibroblasts are involved in contraction [23]. Myofibroblasts are characterized by the expression of αSMA [24]. Fig. 5A and B are representative images of αSMA-expressing cells analyzed by flow cytometry. The data shows that the media from burn patient PBMCs significantly increased the percentage of the positive cells compared to
controls (8.33 ± 2.16% vs 4.37 ± 0.44%, respectively) (Fig. 5C). The result indicates that burn patient PBMCs may release some cytokines, which promote the fibroblast differentiation into contractile myofibroblasts.

3.5. Conditioned media from burn patient PBMCs regulated protein production of TGF-β1, CTGF and DCN in dermal fibroblasts

ELISA was performed to determine the protein level of TGF-β1, CTGF and DCN produced by dermal fibroblasts treated with conditioned media from PBMCs. Compared to control media, the media from burn patient PBMCs was found to enhance the production of fibrotic factor CTGF (608.7 ± 89.7 vs 243.5 ± 39.6 pg/ml) and TGF-β1 (721.1 ± 34.5 vs 250.3 ± 75.1 pg/ml) and decrease the antifibrotic factor DCN (1101.2 ± 58.2 vs 1493.5 ± 61.7 pg/ml) significantly (Fig. 6), which is consistent with the RT-qPCR results.

4. Discussion

In this study, a novel subpopulation of PBMCs expressing CD14 ‘COL-1’ was determined in peripheral blood at the early stage of major burn injured patients, who developed significant HTS following their wound healing. Some of these cells coexpress CD45 and LSP-1, which were demonstrated the markers of fibrocytes [25], and some coexpress CD45 and CD204. CD204 is also known as macrophage scavenger receptor 1, a M2 marker in tissue. This subpopulation was speculated as a mixture of fibrocytes and immature M2 macrophages. Since the technical limitation in the isolation of the specific subpopulation, total patient PBMCs were used to test their effect on fibroblast functions in vitro. We found they are able to up-regulate fibrotic functions of dermal fibroblasts, maybe through the roles of profibrotic factors, demonstrating a possible role in the hypertrophic scarring.

The process that drives fibrotic wound healing are complex, and recently there is increasing evidence that blood-borne cells play an important role in the wound healing process and scar formation. Likely the phenotypes of activated blood-borne cells and the cytokines released from them are related to the severity of injury and the phases of wound healing, which may doom the outcomes of the wound healings. Multipotential hematopoietic stem cells respond to burn injury and differentiate to myeloid progenitors and further effective cells, such as monocytes, macrophages, fibrocytes, etc., involving in wound healing. We are theorizing that it is a dynamic process and closely links to microenvironment. The two populations of CD14 ‘COL’ and CD14 ‘COL1’ may appear in different phases of wound healing or different microenvironment of normal or fibrotic healing. An influx of peripheral blood monocytes derived from a rapidly dividing pool of cells in the bone marrow in response to injury and inflammation can differentiate into fibrocytes and macrophages. Fibrocytes are reported to be involved in the inflammatory fibrotic processes in such diseases as systemic fibrosis, atherosclerosis, asthma, hypertrophic scarring and keloid formation [26–30]. The phenotype of macrophages that remain in the wound bed likely decides their function in collagen production, angiogenesis and re-epithelialization thus influencing the wound healing process. Recently, we have found a positive correlation between pro-inflammatory
cytokines present in the circulation in the early phase post-injury and burn area [31], which indicates that the severity of injury is closely related to the development of fibrosis. Some studies support the role of circulating fibrocytes in the development of HTS following burn injury [12,17,25,26,32,33] including the fact that fibrocytes can differentiate into fibroblasts and myofibroblasts, produce cytokines that induce collagen deposition and promote angiogenesis. As well, fibrocytes are potent antigen presenting cells that can recruit and activate T cells.

We previously reported that in burn patients with HTS, increased SDF-1/CXCR4 signaling was found prior to treatment with subcutaneous IFNα2b including increased SDF-1 expression in HTS tissue and serum as well as CD14 hiCXCR4 cels in the PBMCs, which was down-regulated after IFNα2b treatment, coincident with enhanced remodeling of their HTS. Different from CD14 CXCR4 cels found in this study, the CD14 hiCXCR4 population of PBMCs was determined in the peripheral blood after the acute phase of wound healing, when the scars had formed [31]. A recent study demonstrated markedly elevated levels of SDF-1 in the plasma, blister fluids, hair follicles and blood vessel endothelium in the wounds of burn patients. Also, there were an increased number of fibroblasts expressing SDF-1 observed in the healing dermis after burn injury in rat, swine and human skin [34]. These findings indicate the importance of chemokines in the pathogenesis of HTS by recruiting circulating blood cells through the SDF-1/CXCR4 axis and their contribution to the expansion of the fibroblast/myofibroblast population in HTS.

The Toll-like receptor family appears to play a fundamental role in pathogen recognition and activation of innate immunity by recognizing pathogen-associated molecular patterns and mediating the production of cytokines necessary for the development of effective immunity [35,36]. It has been reported that CXCR4 is not only involved in triggering chemokine pathways, but is involved in LPS binding and signaling [37]. The co-clustering of CXCR4 with other LPS receptors such as TLR4, brings them in close proximity and appears to be crucial for LPS signaling, suggesting that CXCR4 is a functional part of the multimeric LPS “sensing apparatus”. Thus, TLR4 interacts with CXCR4 and the “LPS-sensing apparatus”, contributing to and possibly augmenting its signaling [38]. In our study, over 70% of this cell subset expresses CXCR4 and TLR4, suggesting that SDF-1/CXCR4 signaling and the TLR4 pathway are involved in the activation and recruitment of this PBMC subpopulation following burn injury.
Fig. 5 – Effect of conditioned media from PBMCs on fibroblast differentiation into myofibroblasts. Dermal fibroblasts were cultured with conditioned media from PBMCs of four burn patients and six controls (P-PBMC or C-PBMC). Then the treated cells were stained for αSMA and analyzed by flow cytometry. (A and B) Representative flow cytometry images of cells. (C) Summary of the number of cells expressing αSMA. *P < 0.01.

Fig. 6 – Effect of conditioned media from PBMCs on the production of CTGF, TGF-β1 and DCN in dermal fibroblasts. Dermal fibroblasts were treated with conditioned media from PBMCs of four burn patients or six controls (P-PBMC or C-PBMC) for 24 h and washed, then the remaining cells were continued in culture for another 24 h. The cell supernatant was collected for the determination of TGF-β1, CTGF and DCN by ELISA. The data is displayed as pg/mL normalized by recombinant human TGF-β1, CTGF and DCN standards. *P < 0.01.
Macrophages are also commonly regarded as a key cell type in wound healing, largely for their early inflammatory role\cite{39,40}. As well, it has been recognized that fibrosis is associated with a strong Th2 cytokine milieu in models such as bleomycin- and helminth-induced lung fibrosis. These studies suggest that in a Th2-dominated environment, macrophages are polarized to an alternatively activated (M2) phenotype. Similarly, after burn injury, a systemic Th2 response and a strong Th2 cytokine milieu were seen in burn patients\cite{41}. Nonetheless, convincing evidence that can establish the mechanism between macrophage/monocytes and fibroblasts, which leads to HTS after burn injury, is still lacking\cite{42}.

It is reported that some overlap exists between fibrocytes and macrophages in terms of their functions and surface markers\cite{27}. For example, Mathai et al. found the blood of patients with systemic sclerosis complicated with lung fibrosis contains precursors common to both M2 macrophages and fibrocytes\cite{43}. In response to IL-1β, cultured human fibrocytes increase production of IL-6, IL-8, CC-chemokine ligand (CCL) 3 and CCL4, which would promote inflammatory cell recruitment. Fibrocytes also respond to IL-1β by increasing IL-10 production, which could decrease inflammation and initiate the transition to repair and remodeling by increasing the cell surface expression of leukocyte adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and leukocyte trafficking. Fibrocytes can also secrete paracrine factors such as platelet-derived growth factor (PDGF) and TGF-β1, which induce the differentiation of fibroblasts to myofibroblasts in culture\cite{44}.

5. Conclusion

Our data shows that a novel subpopulation of PBMCs expressing the markers of fibrocytes and M2 macrophages emerges in the circulation following major burn injury and these activated cells may recruit into the wounds under the direction of the chemokine signaling (i.e. SDF-1/CXCR4) that modulate the functions of resident connective tissue cells (i.e. fibroblasts) to fibrosis by producing profibrotic cytokines and mediators. The severity of injuries decides the activation of blood-borne cells in burn patients, which may represent a novel biomarker increasing the risk of hypertrophic scarring. To precisely address which factors released from this novel subpopulation of PBMCs play the critical role in the formation of HTS, observe the dynamic variation of the blood-borne cells responding to varying severity of injuries, and purify the special cell population from circulating PBMCs will be our future directions.

Acknowledgements

This work was supported by the Canadian Institutes of Health Research, the Alberta Heritage Foundation for Medical Research, and the Firefighters’ Burn Trust Fund of the University of Alberta.

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Funding source

University of Alberta Hospital Foundation (Alberta, Canada, RES00012355), Firefighters’ Burn Trust Fund (Edmonton, Canada, D000000520) and Canadian Institutes of Health Research (Canada, G118160818) grant fund this research.

Conflict of interest statement

The authors declare no conflict of interest related to this work.


